Characterisation of novel lung cancer cell lines for immuno-inhibitory markers

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Introduction

The full power of immune system, leads to the elimination of cancerous tumour cells and prevents the development of malignancy. Tumour cells express immunogenic peptides, due to mutation which are recognised as foreign by T-cell and B-cells. However cancer cells can develop mechanisms to escape immune elimination (Hanahan & Weinberg 2011) such as HLA down regulation which can limit peptide expression and decrease immunogenicity and upregulation of PD-L1 which inhibit the action of T-cells, B-cells and macrophages.

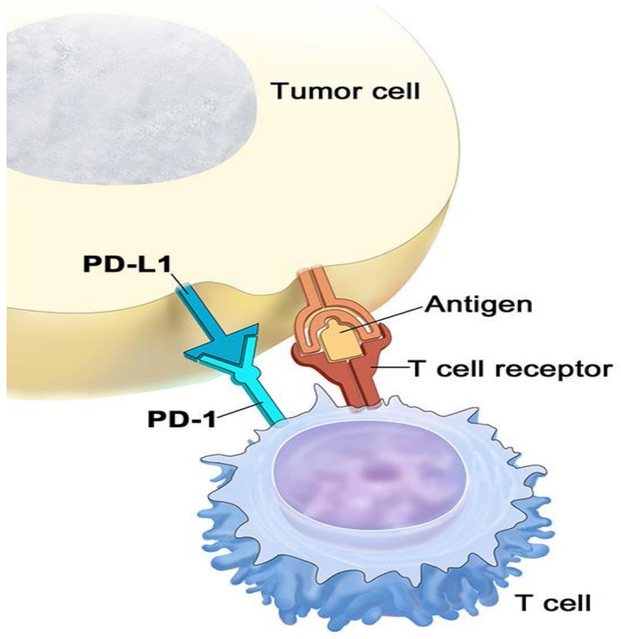


Figure 1: PD-L1/PD-1 binding inhibits T-cell killing of tumour cell (NCI/Winslow 2015).

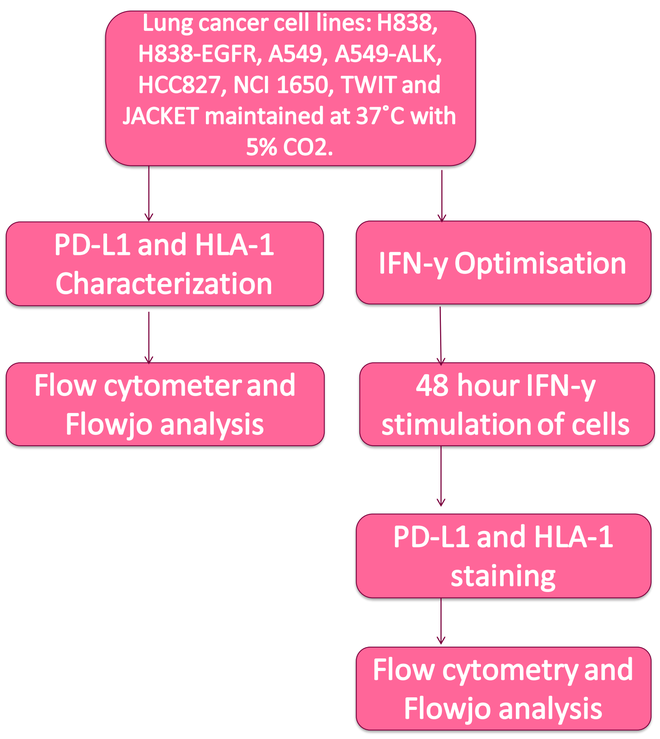
Hypothesis

It was hypothesized PD-L1 and HLA-1 are upregulated in lung cancer cell lines (H838, H838-EGFR, A549, A549-ALK, NCI 1650, HCC 827, TWIT & JACKET) and can be modulated by IFN-γ

Aims

The aim of this study was to quantify the percent expression of PD-L1 and HLA-1 in lung cancer cell lines in the presence and absence of IFN-γ.

Methods



Materials

Media used: DMEM (Gibco) supplemented with 10% fetal calf serum and 1% Penicillin-streptomycin. RPMI (Gibco) supplemented with 10% fetal calf serum and 1% Penicillin-streptomycin. 50:50 mix of the WIT-P medium and Wit-T (Cellaria). Renaissance medium (RETM) (Cellaria) with 4% Hyclone serum and 3% RETM supplement.
Antibodies: APC anti-human CD274 (B7-H1, PD-L1) antibody (Biolegend). APC Mouse IgG2b, κ Isotype Control Antibody (Biolegend). FITC Mouse Anti-Human HLA-ABC (BD bioscience). FITC Mouse IgG1, κ Isotype Control (BD bioscience).
Recombinant Human IFN-γ (carrier-free) (Biolegend)

Results

PD-L1 characterisation in lung cancer cell lines

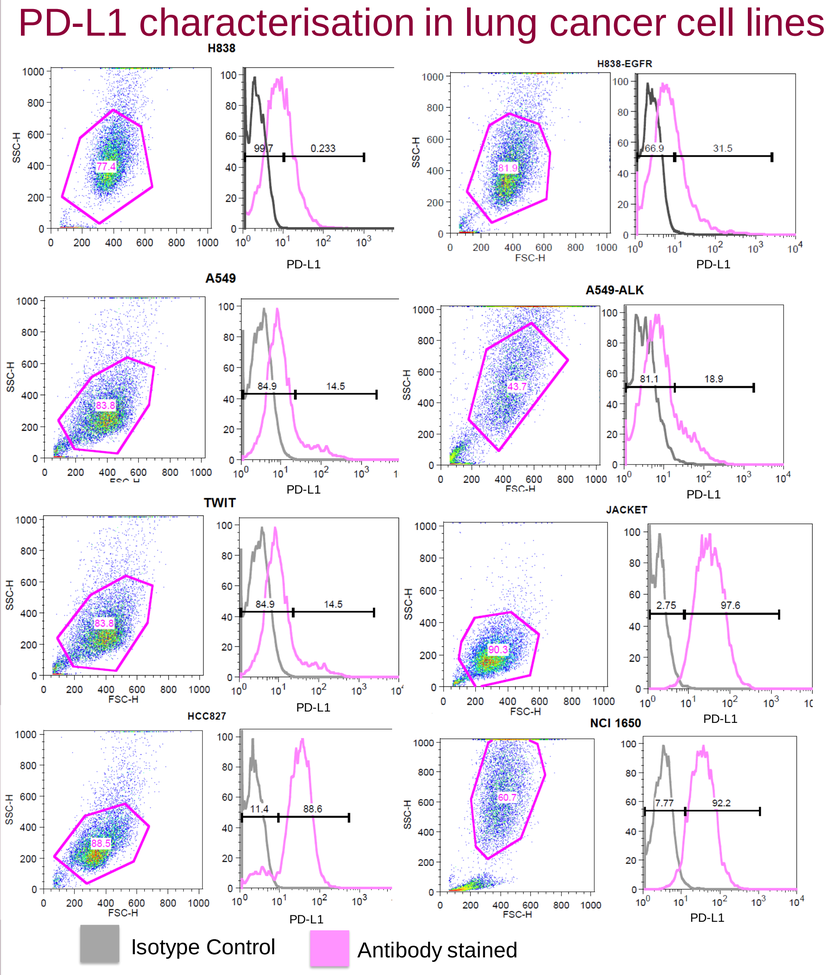
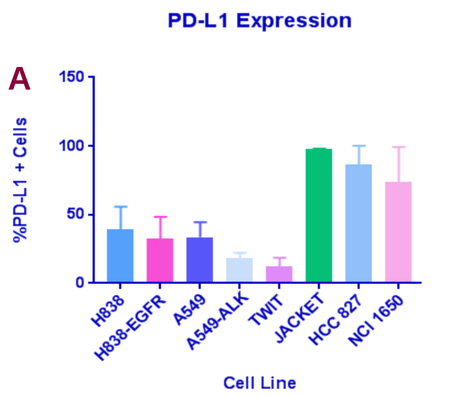


Figure 2: Representative flow cytometric gating strategies used for PD-L1 analysis in cell lines H838, H838-EGFR, A549, A549-ALK, TWIT, JACKET, HCC 827 and NCI 1650 respectively.



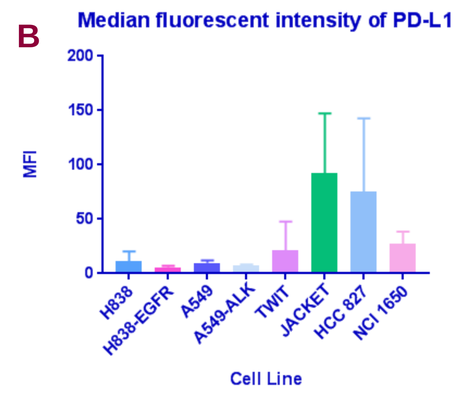
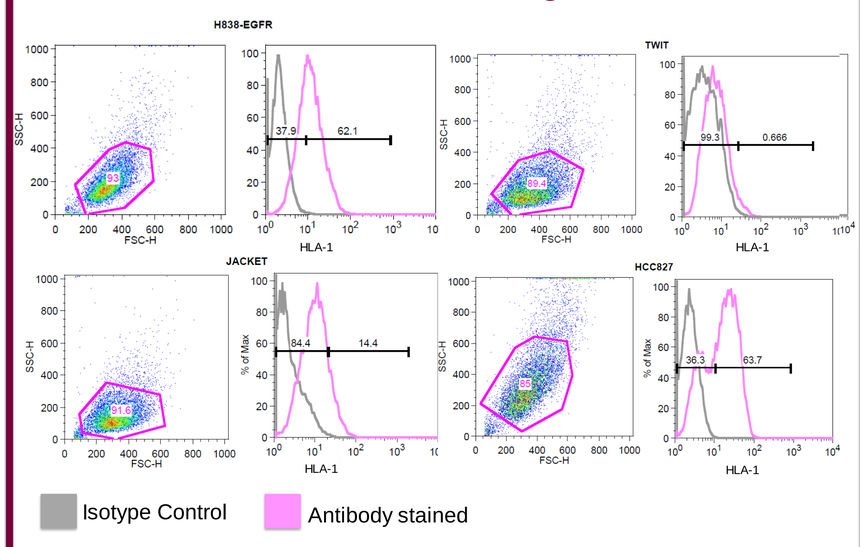


Figure 3A: Mean and SD of PD-L1 cell percentage expression across lung cancer cell lines varies in the absence of IFN-γ. n=3 for independent repeats. **3B:** Median fluorescent intensity (MFI) of PD-L1 expression

NCI 1650, JACKET and HCC 827 present with high PD-L1 expression. H838, H838-EGFR and A549 express a more moderate percentage of PD-L1 whilst TWIT and A549-ALK have a low level of PD-L1 expression

HLA-1 characterisation in lung cancer cell lines



HLA-1 characterisation in lung cancer cell lines continued

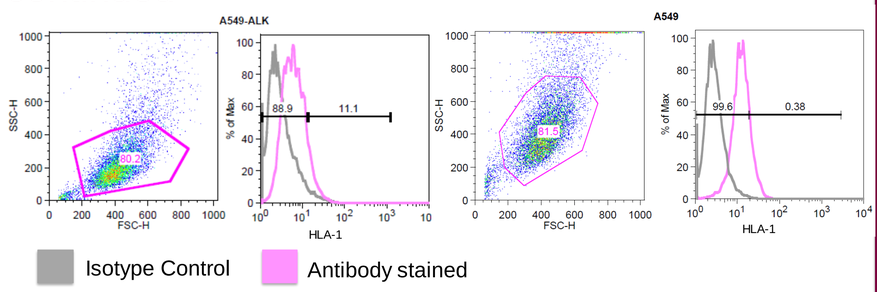


Figure 4: Flow cytometric gating strategies used for HLA-1 analysis of cell lines H838-EGFR, TWIT, JACKETS, HCC 827, A549-ALK and A549 respectively.

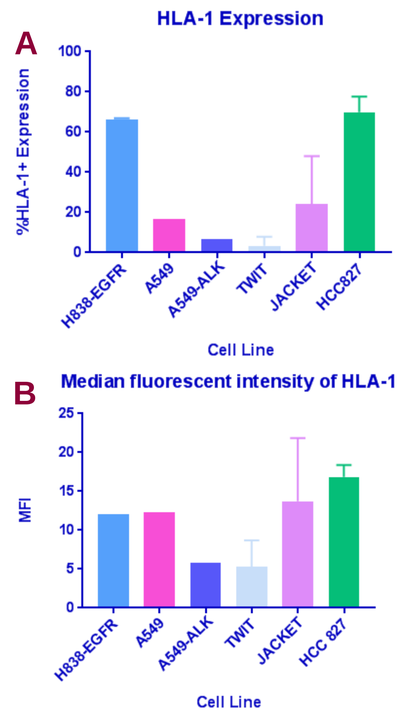


Figure 5A: HLA-1 cell percentage expression in unstimulated lung cancer cell lines. levels. **5B:** MFI of HLA-1 expression

H838-EGFR, HCC 827 present with high HLA expression whilst TWIT, JACKET, A549 and A549-ALK cell lines express HLA-1 at lower levels

Modulating cell surface antigens with IFN-γ

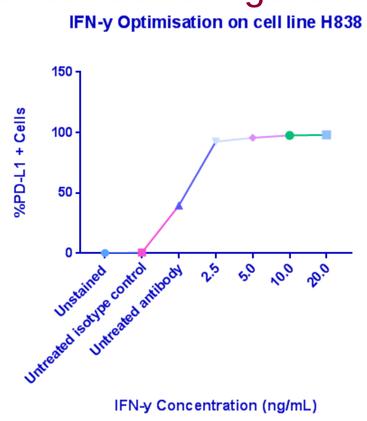


Figure 6: Optimisation of IFN-γ to find the concentration which would maximise PD-L1 upregulation compared to untreated controls.

10ng/mL was determined as the optimum due to the plateauing after 10ng/mL

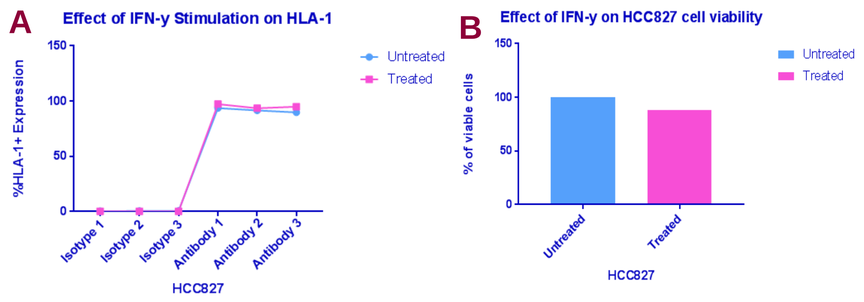


Figure 7A: IFN-γ stimulation of cell line HCC 827. HLA-1 has been upregulated by IFN-γ. Following a student T test statistical analysis it was revealed that there was no significant difference between IFN-γ treated and untreated HLA-1 expression. **7B:** The effect of IFN-γ viability of HCC 827 cells.

Conclusion & future work

- IFN-γ increases PD-L1 expression
- Treat all cell lines with IFN-γ with repeats
- Assess the effect of IFN-γ on cell viability for all cell lines
- Treat cell lines with a combination of IFN-γ and TNF-α

References

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